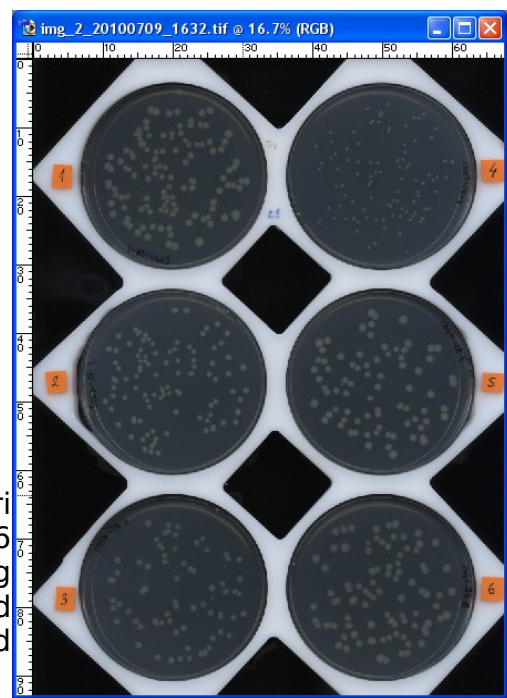# Colony growth tracking

The purpose of the applications described below is first to prepare the raw images for convenient image analysis, then to analyze each Petri dish and finally to reconstruct the statistics of appearance time and growth rates of all dishes that belong to the same strain/condition.

Image analysis

1. Pre-Processing: Each scanner produces an image of up to 6 Petri dishes. Each Petri dish is analyzed separately (because each dish may contain a different condition). The preprocessing stage purpose is to align all the images of a specified scanner, and to separate the 6 Petri dishs. It is assumed that the raw images are similar to the example shown below, namely that each scanner monitors up to 6 standard Petri dishes, that are placed in a fixed position on the scanner.

Typical raw image of 6 standard Petri dishes on one of the scanners. The 6 plates are placed inside a "Scanning board" – a rigid board with 6 round slots that keeps the plates in a fixed position on the scanner.

```
PreparePictures(DestDirName, board, plateVec,
SourceDirName,LogFile,startDate)
```

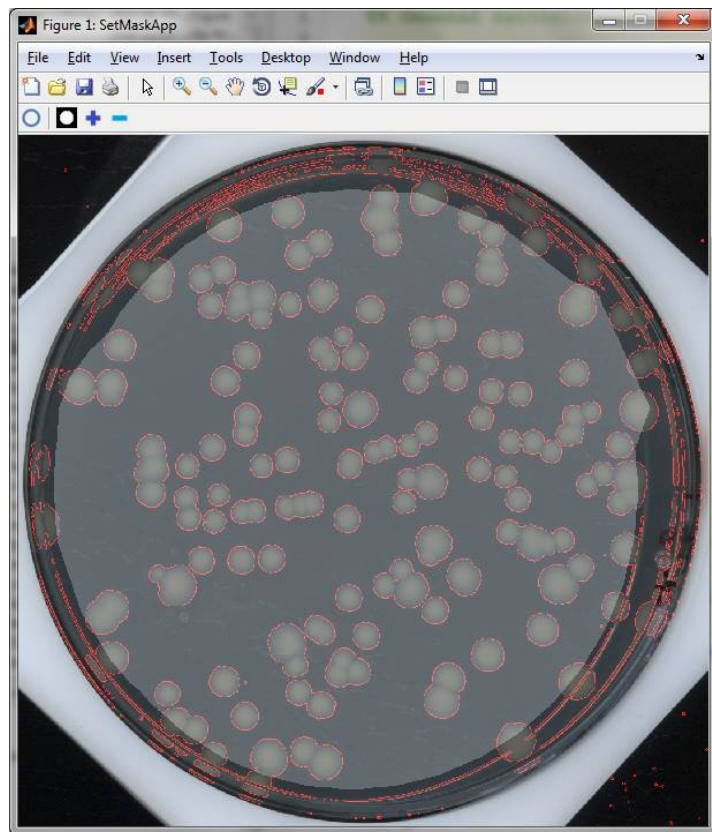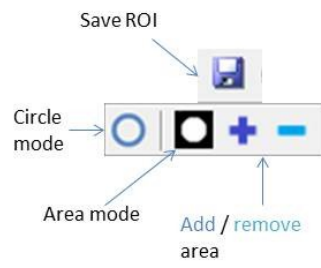| Purpose	Cut the image into 6 separate Petri dishes	
Parameters	`DestDirName`	Destination library. The resulting files will be placed in that library.
	`board`	The number of the "Scanning board"
	`[plateVec]`	An array of the wanted Petri dishes (default – all 6)
	`[SourceDirName]`	Original images library (if not mentioned, you will be asked to choose)
	`LogFile`	The log file of the process. If this argument is not empty string the start time of the experiment will be calculated by the first line of this file.
	`startDate`	If previous argument is an empty string this string will be evaluated to the starting time of the experiment. Please use the 'yyyy/mm/dd HH:MM:SS' format
Description	The software aligns the images, finds the Petri dishes in the image, and cuts each Petri dish. The images of the separate Petri dish are placed in `DirName_#\Pictures` (where # is the number of the dish). The images are named `P#_*****.tif` (where # is the number of the dish, and ***** are 5 digits representing the time in minutes passed from the beginning of the experiment).	
Example	`PreparePictures('D:\scans\Images',2, [2 3 5], 'F:\scans\Images');`	

```
createBoardDefinitionFile(Img, Board)
```

Purpose	Defining a new "Scanning board". A "Scanning board" is a rigid board with 6 round slots for the Petri dishes, as shown in the picture above.	
Parameters	`Img`	A grayscale image of the board, taken by the scanner at 300 dpi (as shown above)
	`Board`	The number of the "Scanning board"
Description	Copy an existing "Scanning board" file and rename it to the number of the new board: `BoardDefinitionFile_##.mat`, and then activate this function. The function finds the appropriate parameters for the locations of the Petri dishes on that board, and saves it. The function does up to 15 trails to find a good match, and can be activated over and over again till the circles of the plate locations match well with the image.	
Example	`createBoardDefinitionFile (GrayScaleImage, 23);`	

2. Analyzing the images: The purpose of this stage is to identify colonies in the images of the dishes, and to track them in time. This is done for each Petri dish separately using `TimeLapse` or sequentially for several Petri dishes using `TLAllPlates`. Before running the TimeLapse please determine the ROI (Region Of Interest) to be considered when analyzing the colonies using `setMaskApp` (This should be done to each plate seperatley).
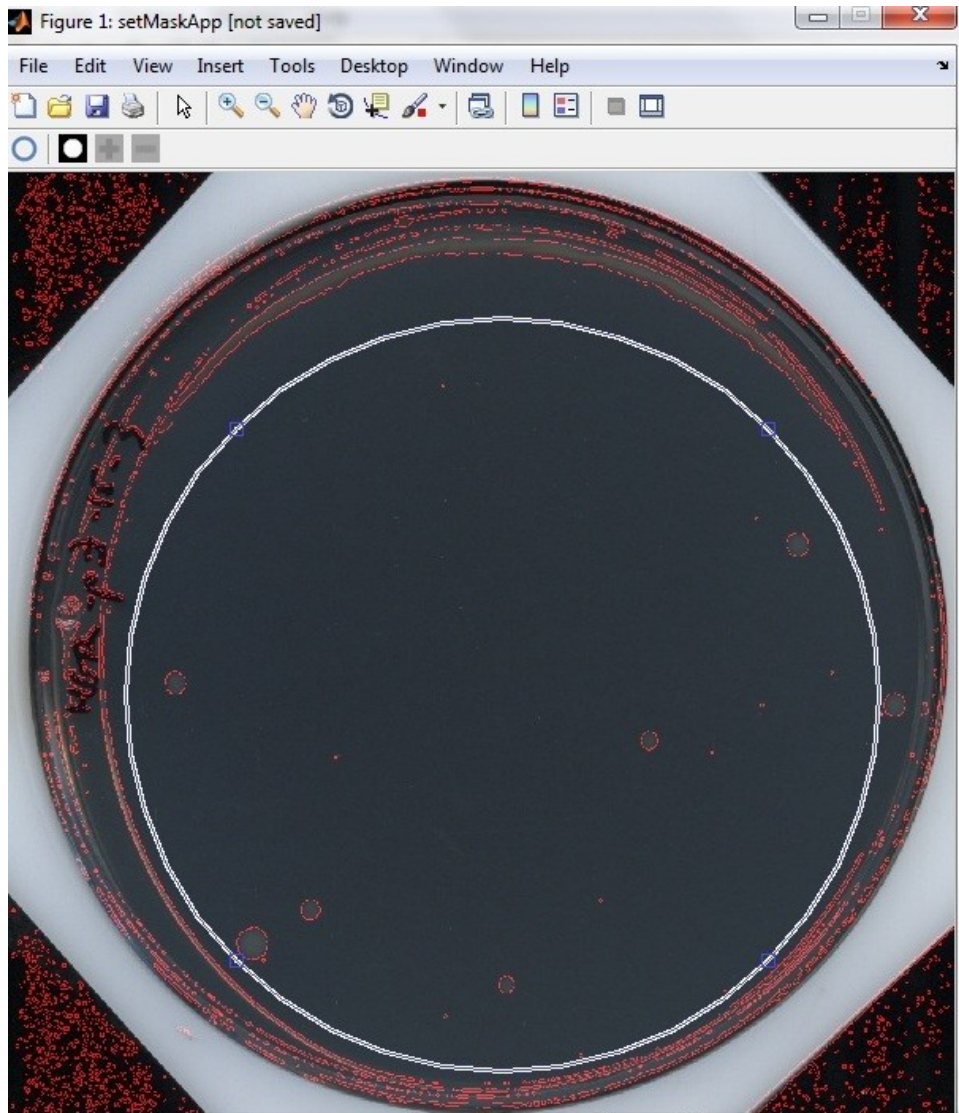
setMaskApp(DirName, TH)

Purpose	A tool for determine the ROI of specific Petri dish.
Parameters	`DirName` — The root directory of the Petri dish (full path)
	`[TH]` — A threshold for identifying the colonies in the grayscale image. Use ShowLastStretchedHist.m to figure it up. (Default is 0.2, that fits Epson scanners with LB Agar dishes plated with *E.Coli*.our scanners (most of the time)).
Description	Displaying the Petri dish's last image and specify what are the predicted colonies in the image. The user can then select the wanted ROI using circled roi or by building its own binary image by adding or removing polygonal roi's.

Important: When analyzing a Petri dish with small number of colonies please build the mask around the colonies (like in the image below). |
| Example | `setMaskApp`('D:\scans\Images1') |
| Result | |

Save ROI

Circle mode

Area mode

Add / remove area

example for circ roi mode:

Not

```
TimeLapse(DirName, Description,TH)
```

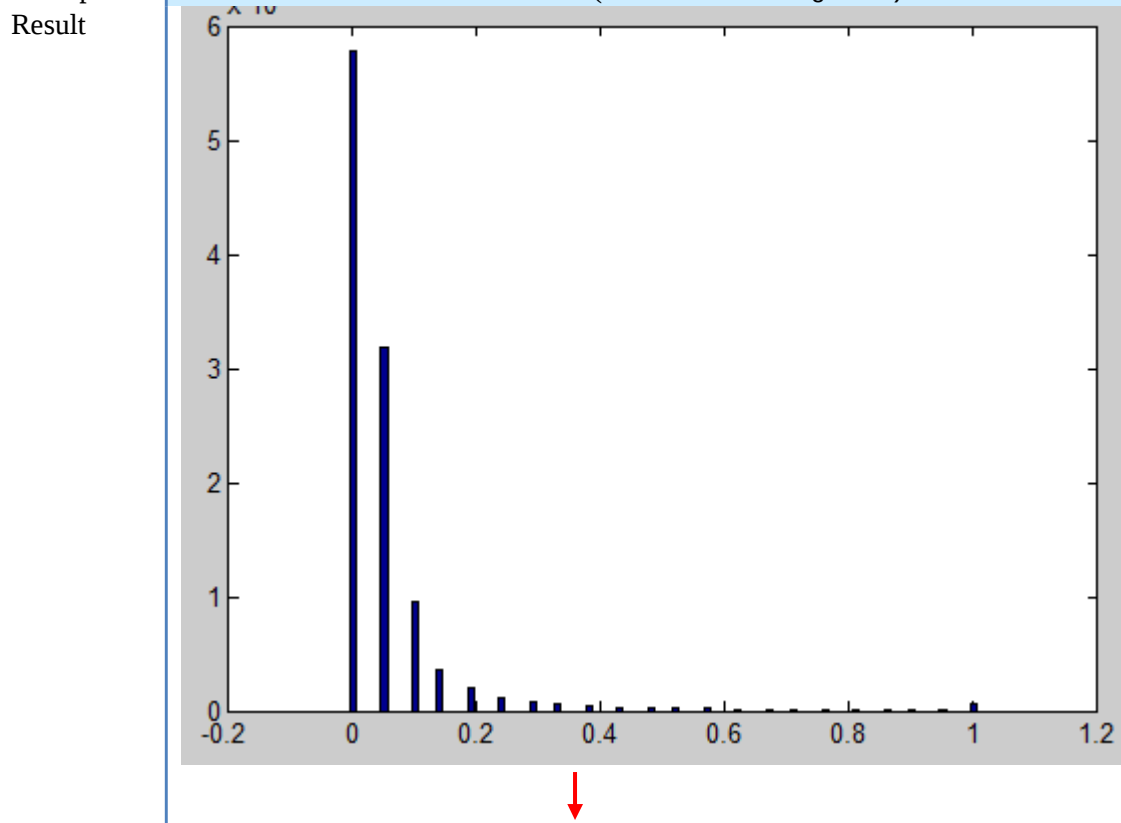| Purpose	Analyzing one Petri dish	
Parameters	DirName	Library name of the images. (the same as `DirName_#` in bullet 1)
	[Description]	Text description of the experiment (that will appear in the headings of the graphs)
	[TH]	A threshold for identifying the colonies in the grayscaled image. Use ShowLastStretchedHist.m to figure it up. Default is 0.2, that fits both our scanners (moost of the time).
Description	The application creates three sub folders under the directory `DirName` (in addition to the subfolder created earlier `\Pictures`). The labeled images are under `DirName\LRGB` and called L#_##### according to the image it was created from. `DirName\TmpCleanImg` places the images after processing as jpg for a quicker movie rendering and for quicker display of the plate. This sub folder can be erased if needed. `DirName\Results` places the results of the analysis, the	

Example	PlateMovie.avi and the log file. `TimeLapse('D:\scans\Images1', 'description 1');`

TLAllPlates(DirVec, DescriptionVec)

Purpose	Analyzing all Petri dishes at once (please add a word on how it separates different conditions)	
Parameters	`DirVec`	`{'DirName1';'DirName2';'  ...  '}` a vertical cell array of folder names
	`[DescriptionVec]`	`{'Plate1  description'; '';…}` a vertical cell array of descriptions
Description	The application performs `TimeLapse` for all the `DirNames` in `DirVec`. Each Petri dish is analyzed separately, and the analysis results are stored in separate directories.	
Example	`TLAllPlates ({'D:\scans\Images1'; 'D:\scans\Images2'}, {'description 1'; 'description 2'});`	

ShowLastStretchedHist(DirName)

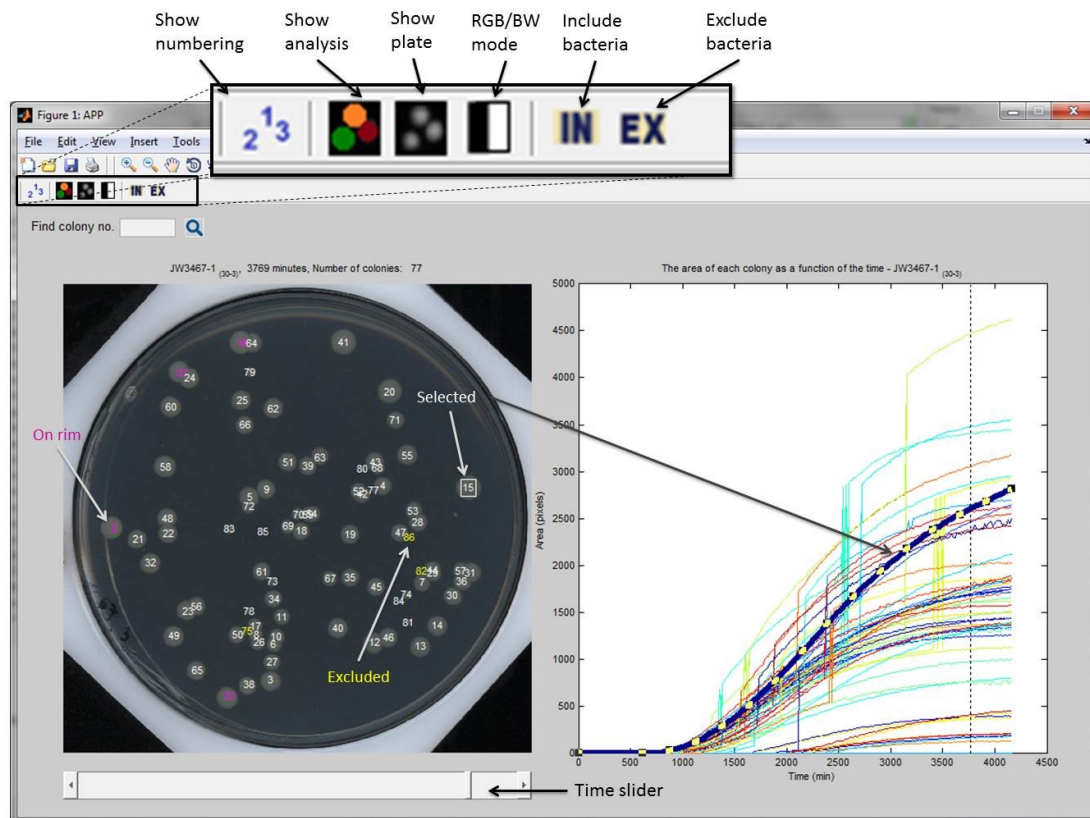|	
Purpose	A tool for determine the TH or the colonies identification in the grayscale image
Parameters	`DirName` The root directory of the Petri dish (full path)
Description	This tool shows the last image's stretched histogram. By this histogram one can determine the gray values of the colonies. This value will be a parameter for TimeLapse (if not given, the default is TH=0.2).
Example	`ShowLastStretchedHist('D:\scans\Images1')`
Result	

Data analysis

The purpose of this stage is to extract the statistics of appearance time and growth rates of all dishes that belong to the same strain/condition. Some functions works on one Petri dish (e.g. ScanLagApp, ShowAreaGraph, ShowPlate, ~~WhichColony,~~ makePlateMovie, ~~FilterBacteria, set/getPlateCentre~~), while other functions use the data from all Petri dishes with the same condition (e.g. AddHistograms, plotDeathCurve, GetAppearanceTimes, getStatistics).

Major functions list:

ScanLagApp(DirName)

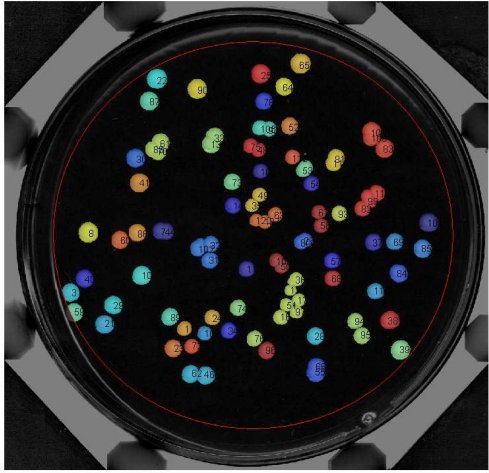|	
Purpose	A tool for investigation of the automatic analysis
Parameters	DirName The root directory of the Petri dish (full path)
Description	Displaying the Petri dish alongside with the graph of the colony size versus time. Using this tool you can associate a colony with its curve on the area graph, you can locate a colony, and exclude analysis defects (or include). Using the time slider you can scroll through the experiment at different times and learn about the development of the colonies. When show analysis in on, the colonies are painted with their colour, similar to the colour of their curves (as shown on ShowPlate). The RGB/BW mode switches between the original plate image and the black and white image after background subtraction. Colour coding of the text IDs of the colonies: The ID number is red for new identified colonies, blue for something identified (dirt) that doesn't appear in the next frame, yellow for manually screened colonies, and purple for colonies that are on the rim of the analyzable area of the plate.
Example	ScanLagApp('D:\scans\Images1')
Result	

Show numbering | Show analysis | Show plate | RGB/BW mode | Include bacteria | Exclude bacteria

ShowAreaGraph(FileDir)

Purpose	Display a graph of the colony size versus time for one Petri dish
Parameters	FileDir The root directory of the Petri dish (full path)
Description	Displaying the area in pixels of each colony in the Petri dish vs. time (in minutes). When colonies merge, their common area is rising together, and it seems like a spike in the area curve.
Example	ShowAreaGraph('D:\scans\Images1')
Result	

ShowPlate(TimeGap,DirName,forMovie)

Purpose	Displaying the Petri dish at the specified time	
Parameters	TimeGap	Time in minutes from the beginning of the experiment
	DirName	The root directory of the Petri dish (full path)

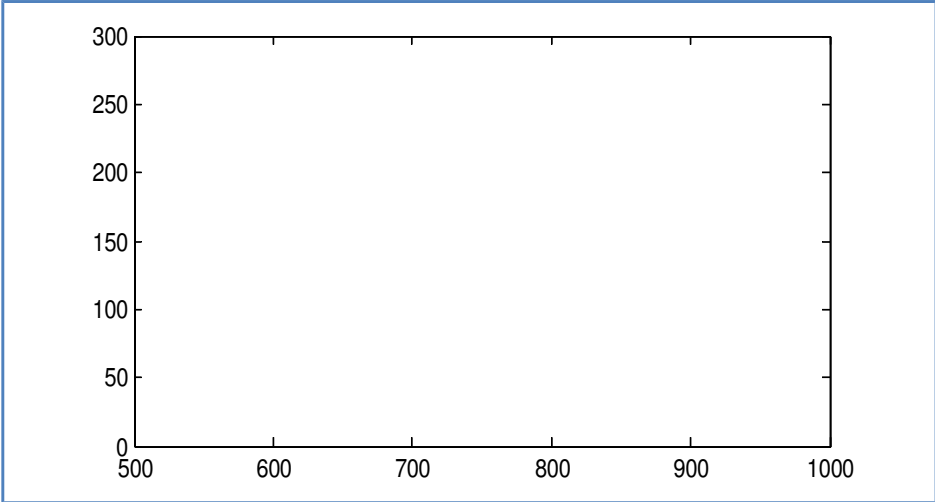|	[forMovie]	Boolean, default - 0. Use 0 to see the plate in a good resolution
Description		Displaying the Petri dish at the specified time, marking the identified colonies with arbitrary colours, and with the colony IDs on top (the colour of each colony is the same as the colour of the curve that represents this colony in the Area vs. Time graph). The ID number is red for new identified colonies, blue for something identified (dirt) that doesn't appear in the next frame, and yellow for manually screened colonies. If the sub-folder `DirName\TmpCleanImg` exists, the function will run faster, because the images after processing are saved there.
Example		`ShowPlate(1993,'D:\scans\Images1',0)`
Result		

exp$_{(s24\,p1)}$ 1993 minutes, Number of colonies: 93

F = makePlateMovie(DirName)

Purpose		Creating a movie of the colonies' growth in time, for a specified dish
Parameters	DirName	The root directory of the Petri dish (full path)
Returns	F	A structure of all the frames
Description		The function uses `ShowPlate` to display all frames of the Petri dish. The result is `DirName\Results\PlateMovie.avi`. NOTE: A)Matlab captures the figure as it is displayed on screen to create a frame, therefore while rendering a movie you cannot use the computer. B) The resolution of a single frame is better than the resolution of the movie.
Example		`makePlateMovie('D:\scans\Images1')`
Result		`DirName\Results\PlateMovie.avi`

[TimeAxis, Histogram] = AddHistograms(DirVec, bin)

Purpose		Creates a histogram of appearance times
Parameters	DirVec	A vertical cell array of the directory names
	bin	Size of bins in minutes
Returns	TimeAxis	An array of the bins
	Histogram	Amount of colonies in each bin
	plotHist	Ploting the histogram or not
Description		Uses `GetAppearanceTimes` to create a histogram
Example		`[TA,Histo]= AddHistograms ({'D:\scans\Images1'; 'D:\scans\Images2'},30)` `figure; bin(TA, Histo)`

Result

[Time, DC] = plotDeathCurve(DirVec,ShowPlot)

Purpose	Plots 1-CDF representation of the distribution	
Parameters	DirVec	A vertical cell array of the directory names
	ShowPlot	0 – don't show (default) 1 - show
Returns	Time	An array of the bins
	DC	1-CDF
Description	Uses GetAppearanceTimes to create a histogram	
Example	[TA,DC]= plotDeathCurve ({'D:\scans\Images1'; 'D:\scans\Images2'},0) figure; semilogy(TA, DC)	

Result

[TimeAxis,TotalAppearanceTime]=GetAppearanceTimes(DirVec)

Purpose	Getting the appearance time all colonies in the experiment	
Parameters	DirVec	A vertical cell array of the directory names
Returns	TimeAxis	Times of the images
	TotalAppearanceTime	An array of appearance times
Description	Adds the appearance time of each colony into an array	
Example	[TA,AppTimes]=GetAppearanceTimes({'D:\scans\Images1'; 'D:\scans\Images2'})	

```
Stat = getStatistics(TimeAxis, TotalAppearenceTime)
```

Purpose	Getting statistical parameters on the experiment	
Parameters	`TimeAxis`	Times of the images
	`TotalAppearenceTime`	An array of appearance times
Returns	Stat	A structure that contains: total – total amount of bacteria, Avg – average, std – standard deviation, skw – skewness, max – histogram peek, median and stdMed – median and standard deviation from the median.
Description	Calculates all statistical parameters from the array of appearance times.	
Example	`Stat = getStatistics(TA, AppTimes)`	